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HOME-GROWN GRAIN LEGUMES IN POULTRY DIETS

DOCTORAL THESIS

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ACADEMIC DISSERTATION

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“There is no substitute for hard work – Thomas Edison”

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following journal articles, which are referred to by the Roman numerals in the text I – IV. The papers are reprinted with the permission of the publishers.

- I** Koivunen, E., Tuunainen, P., Valkonen, E., Valaja, J., 2015. Use of semi-leafless peas (*Pisum sativum* L) in laying hen diets. *Agricultural and Food Science*, 24: 84–91.

- II** Koivunen, E., Tuunainen, P., Valkonen, E., Rossow, L., Valaja, J., 2014. Use of faba beans (*Vicia faba* L.) in diets of laying hens. *Agricultural and Food Science*, 23: 165–172.

- III** Koivunen, E., Talvio, E., Valkonen, E., Tupasela, T., Tuunainen, P., Valaja, J., 2015. Use of semi-leafless peas (*Pisum sativum* L) with enzyme addition in diets for broilers. Submitted

- IV** Koivunen, E., Tuunainen, P., Rossow, L., Valaja, J., 2015. Digestibility and utilization of faba bean (*Vicia Faba* L.) diets in broilers. *Acta Agriculture Scandinavica, Section A, Animal Science*. DOI:10.1080/09064702.2015.102085

- V** Koivunen, E., Partanen, K., Perttilä, S., Palander, S., Tuunainen, P., Valaja, J., 2015. Digestibility and energy value of pea (*Pisum sativum* L), faba bean (*Vicia faba* L) and blue lupin (narrow-leaf) (*Lupinus angustifolius*) seeds in broilers. Submitted

CONTRIBUTIONS

The contributions of all authors in the original articles of this thesis are presented in the following table

	I	II	III	IV	V
Planning the experiment	EV, ET, JV	EK, EV, JV, PT,	ET, EV, JV, TT	EK, JV, PT	KP, SPa, SPe, JV
Conducting the experiment	ET, EV, JV	EK, JV, PT	ET, EV, JV, TT	EK, PT	KP, SPa, SPe, JV
Conducting the autopsies	-	LR	-	LR	-
Data analysis	EV, EK	EK, EV	EK, ET, JV	EK, JV	KP, SPa, SPe, JV, EK
Calculating and interpreting the results	EK, EV, JV,	EK, EV, JV,	EK, ET, JV, PT, TT	EK, JV	EK, KP, PT, SPa, SPe, JV
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ABSTRACT

The aim of this thesis was to find alternative domestic protein sources for imported soybean meal (SBM). Four experiments (Papers I – IV) were conducted to evaluate nutritive value and to find appropriate inclusion levels of pea (*Pisum sativum* L.) and faba bean (*Vicia faba* L.) (FB) seeds in poultry diets. The inclusion levels tested for pea (cv. Karita) were 100, 200 and 300 g/kg in laying hen diet (I) and 150, 300 and 450 in broiler diet (III) and for FB (cv. Kontu) 50 and 100 g/kg in laying hen diet (II) and 80, 160 and 240 g/kg in broiler diet (IV). The effect of an enzyme cocktail including xylanase (XLS), amylase (AMS) and protease (PRT) for improving the nutritive value of wheat-pea diets was also investigated (III). The fifth experiment (Paper V) was conducted to determine the apparent metabolizable energy (AME) value and the coefficients apparent ileal digestibility (CAID) of nutrients of the seeds of two pea cultivars (cv. Karita and cv. Sohvi), two FB cultivars (cv. Kontu and cv. Ukko), and one bluelupin (*Lupinus angustifolius*) cultivar (cv. Pershatsvet) in broilers. The nitrogen-corrected AME value and the CAIDs of nutrients of FB diets in broilers were also studied (IV).

The crude protein (CP) content of grain legumes (g/kg dry matter (DM) basis) ranged from 199 (pea cv. Sohvi) to 318 (FB cv. Kontu). For lupin CP content was 223 g/kg DM. Compared to SBM, grain legumes had at least moderate lysine content, but they were deficient in methionine. FB (cv. Kontu) seeds had a high tannin (13.7 g/kg DM, III) and vicine + convicine (V + C) (9.9 g/kg DM, mean of II and III) contents.

Pea inclusion up to 300 g/kg had no effect on egg production or egg quality (I). Broilers' growth improved slightly by pea inclusion of 150 g/kg due to higher essential amino acid contents of the diet. Pea inclusion 150 g/kg improved the feed conversion ratio (FCR) of birds on unsupplemented diets, but had no effect when enzymes were used. Pea inclusions 300 and 450 g/kg had no effect on growth or FCR (III). The use of enzyme cocktail improved the nutritive value of wheat- pea diet as demonstrated by an improved performance of broilers (III).

FB inclusion (control vs. FB diets) decreased egg weight and tended to increase the mortality of the hens (II). Egg mass production decreased and FCR increased when FB proportion increased (from 50 to 100 g/kg). FB inclusion had no effect on egg quality. Broilers' body weight, body weight gain, and feed consumption decreased and FCR improved in a linear manner along FB inclusion (IV). FB had no effect on broiler mortality.

The CAID of protein was higher in peas and lupin than in FB cv. Kontu (V). However, there was no difference in the CAID of protein between FB cv. Ukko and other legumes. Most of CAID values of amino acids (AA) followed the pattern shown by the CAID of protein. AAs in peas were well digested (≥ 0.768) except of moderate digested cysteine in Karita (0.671). The AAs were at least averagely digested in FBs (≥ 0.714) with the exception of cysteine, which was poorly digested (≤ 0.516). However, all nutrients of the diets including FBs up to 240 g/kg were well digested (IV). The AAs were at least averagely digested in lupin (≥ 0.763). The AME for pea cv. Karita (13.8 MJ/kg DM) was higher than that of pea cv. Sohvi (12.2 MJ/kg DM). FBs cv. Kontu and Ukko had a lower AME value compared to pea cv. Karita (≤ 12.4 MJ/kg DM). Lupin had the poorest AME (7.0 MJ/kg DM).

In conclusion, grain legumes can partially replace SBM in poultry diets. However, they replace also cereal in the diets. Grain legumes are a good source of lysine in the diet. Peas (cv. Karita) can be used at least up to 300 g/kg in laying hen diets (I) and 450 g/kg in broiler diets (III). The use of tannin and V + C containing FB (Kontu) is recommended to be limited to the content of 50 g/kg in laying hen diets (II) and 160 g/kg in broiler diets (IV).

Keywords: broiler, digestibility, faba bean, laying hen, lupin, pea

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ABBREVIATIONS

AA	amino acid
AME	apparent metabolizable energy
AME _N	nitrogen-corrected apparent metabolizable energy
AMS	amylase
ANF	anti-nutritional factor
CAID	coefficient of apparent ileal digestibility
CP	crude protein
DM	dry matter
EAA	essential amino acid
FCR	feed conversion ratio
IICP	Illinois ideal chick protein
NEAA	non-essential amino acid
NSP	non-starch polysaccharide
PRT	protease
SBM	soybean meal
V + C	vicine and convicine
XLS	xylanase

1 INTRODUCTION

Since the European Commission's ban of the use of meat-bone meal and its by-products in 2001 (Regulation EC 999/2001), imported soybean meal (SBM) has been the main protein source used in poultry diets in Europe. The price formation of SBM in a market is dependent on supply and demand. However, because SBM is a by-product of oil extraction from soybeans, the cost of SBM depends on the price of agricultural commodities on the world market. These prices are influenced by changes in economic growth, consumer product preferences, and weather conditions (Jezierny et al., 2010). Soybean prices are highly volatile, but an upward trend is detectable. Furthermore, the supply of non-genetically modified soybeans is diminishing, and thus the related premiums are increasing. The obvious negative impact of expansion of soybean cultivation is the loss of the natural ecosystems of tropical forest (Fearnside, 2001). The focus of this harmful expansion take place in Latin America, especially Brazil, followed by Bolivia and Paraguay where the risk of the soy monoculture is simultaneously increasing (Fearnside, 2001).

In climates where soybean (*Glycine max*) cannot be produced or its production is not economical, there is a strong interest in maximizing the use of locally produced protein sources such as protein-rich turnip rape (*Brassica rapa* L.), oilseed rape (*Brassica napus* L.), peas (*Pisum sativum* L.), faba beans (*Vicia faba* L.), and *Lupinus* species as a substitute for imported SBM. The use of domestic grain legumes adapted to most climatic areas of Europe offers the possibility to improve self-sufficiency in protein-rich feedstuffs (Gatel, 1994), which is of common concern in European countries.

The declared national aim is to improve Finland's domestic self-sufficiency in supplementary protein, which is currently at 15%. Several proposals for action to increase domestic protein production are presented in the roadmap for improving the protein self-sufficiency of Finland published by VTT (2015). Rapes (*Brassica rapa* L and *Brassica napus* L) are well adapted to growing in the climate zone of Finland (Peltonen-Sanio et al., 2013), and rapeseed by-products can partially replace SBM in poultry diets (Naseem et al., 2006; Gopinger et al., 2014). Since rapeseed breeding in the past decades has led to a significant reduction in glucosinolate content, rapeseed by-products have become a valuable source of protein for feedstuffs (Kozłowski and Jeroch, 2014). Canola is the registered name of cultivars of rapeseed (*Brassica napus*), field mustard (*Brassica rapa*), also known as turnip rape, or of brown mustard (*Brassica juncea*), and these cultivars are widely studied for poultry nutrition. Canola meal can be safely used in layer diets at inclusion rates

from 200 to 240 g/kg (Ward et al., 2009; Khajali and Slominski 2012) and in broiler diets at inclusion rates from 170 g/kg to 250 g/kg (Naseem et al., 2006; Gopinger et al., 2014). However, rapeseed meal is the most important supplementary protein feed for cattle in Finland (Huuskonen, 2013), and the production of rapeseed does not cover the domestic demand for protein feeds for non-ruminants.

One of the most effective ways of increasing protein self-sufficiency is to increase the cultivation of legumes (VTT, 2015). The temperate crops peas and faba beans are grown and bred in Finland (Peltonen-Sainio et al., 2009). They are currently minor crops, but due to climate change they are particularly strong candidates for becoming major crops, and their cultivation has already increased during the last decade (Peltonen-Sainio et al., 2009; Peltonen-Sainio et al., 2013). The terms lupin and lupins are widely used to describe the seed or grain of domesticated *Lupinus* species, namely of *L. albus*, *L. angustifolius*, *L. cosentinii*, *L. luteus*, or *L. mutabilis* (Pettersson, 2000). Lupin (blue lupin) (*Lupinus angustifolius*), which is studied in this thesis, as well as *Lupinus* species in general are produced on a very limited scale in Finland (Peltonen-Sainio et al., 2009; Stoddard et al., 2009). However, blue lupin is grown in experiments in southern and northern Finland (Peltonen-Sainio et al., 2009). Lupins are also likely to benefit from climate-induced changes in Finland, and they could represent a valuable addition to the group of nitrogen fixing protein crops in the future (Peltonen-Sainio et al., 2009). The interest in producing blue lupin (*Lupinus angustifolius*) has recently increased.

In Finland, many areas are also favorable for crop-based protein production from legumes (Peltonen-Sainio et al., 2013). Increasing domestic legume cultivation is one way of diversifying the northern crop system, which is dominated by cereals (Peltonen-Sainio et al., 2013). Legumes have an important role in crop rotation due to their ability to fix nitrogen (Stoddard et al., 2009; Jensen et al., 2010). Grain legumes can be grown without nitrogen fertilization though the small amount of nitrogen is usually given to support early growth (Jensen et al., 2010). Of the major cool season grain legumes, faba beans have the highest average reliance on nitrogen fixation for growth. As a consequence, the nitrogen benefit for the following crop is often high, and several studies have demonstrated substantial savings (up to 100 – 200 kg of nitrogen per ha) in the amount of nitrogen fertilizer required to maximize the yield of crops grown after faba beans (Jensen et al., 2010). Growing legumes has a special function in organic farming because of their nitrogen fixing ability (Stoddard et al., 2009). Petroleum is required for the production of nitrogen fertilizer, so growing legumes also reduces oil consumption.

Grain legumes contain considerably less protein than SBM does. Grain legume protein is as good a source of lysine as SBM protein, but methionine is present at lower levels in grain legume protein (Gatel, 1994; Perez-Maldonado et al., 1999; Steenfeldt et al., 2003). This limitation is explained by the AA composition of the main storage protein globulins, whose sulfur AA content is much lower than that of the albumins typical for soybeans (Gatel, 1994). In addition, the apparent ileal digestibility of methionine and cysteine in grain legumes has often found to be low, especially in faba beans (Castell et al., 1996; Perez-Maldonado et al., 1999; Palander et al., 2006). However, considering the protein AA profile of cereals and grain legumes, they complement each other well, and feed grade crystalline AAs in conventional poultry diets may help to alleviate the shortages in the AA composition of grain legumes (Gatel, 1994; Igbasan and Guenter, 1996).

Grain legumes contain a number of anti-nutritional factors (ANF), including condensed tannins, trypsin and protease inhibitors, lectins, alkaloids, saponins, phenolic acids, flatulent oligosaccharides and the pyrimidine glycosides vicine and convicine (V + C) (Dvořák et al., 2006; Jezierny et al., 2010). Among those ANFs most harmful for poultry are condensed tannins, trypsin and protease inhibitors, and particularly V + C (Crépon et al., 2010). Moreover, lectins, saponins, and protease and trypsin inhibitors are all also present in SBM (Jezierny et al., 2010). The ANFs may exert a wide range of different effects on the animals that consume them (Jezierny et al., 2010). The ANFs may impair growth performance, fertility, and the health status of livestock due to a variety of underlying mechanisms (Jezierny et al., 2010). It is well known that nutritive value and ANF contents in all grain legumes generally depend on the cultivar as well as on the growing conditions (Duc et al., 1999; Smulikowska et al., 2001; Crépon et al., 2010).

Condensed tannins are present in faba beans and peas (Marquardt et al., 1977; Bastianelli et al., 1998), while lupins are almost devoid of them (Pettersson, 2000). Due to condensed tannins and specific trypsin and protease inhibitors, the nutritive value of peas and faba beans is often lower than expected (Gatel, 1994). Tannins impair protein digestibility (Gatel, 1994; Crépon et al., 2010). In addition, tannins may have a considerable influence on the grain legume palatability (Reed, 1995; Berger et al., 2003). The primary mode of action of trypsin protease inhibitors consist of inhibiting the secretion of the proteolytic pancreatic enzymes trypsin and chymotrypsin into the intestinal lumen (Gatel, 1994). As a result, losses of endogenous methionine and cysteine – via enhanced secretion of trypsin and chymotrypsin – may inhibit the growth of animals (Belitz and Weder, 1990; Liener, 1994). Dehulling (Marquardt et al., 1977; Ward et al., 1977), autoclaving (Marquardt et al., 1974), micronising (McNab and Wilson, 1974) and different treatments like

consisting in exposure to temperature over 100 °C to organic acids and substrate maturation plus subsequent drying (Dvořák et al., 2006) have decreased the content of condensed tannins. Methods tested by Dvořák et al. (2006) also decreased the content of trypsin inhibitors.

Lectins can bind to receptors of epithelial cells of the intestinal mucosa and disturb digestive processes (Gatel, 1994). In addition, lectins change gut immune function, reduce production of endocrine cells and gut hormones, interfere with the bacterial ecology in the gut lumen, and damage mucosal cells (King et al., 1983). However, there is low lectin activity in faba beans, peas (Gatel, 1994), and blue lupin (narrow-leafed) (*Lupinus angustifolius*) (Pettersson, 2000), which offers advantage over SBM, which need heat treatment to inactive lectins (Pettersson, 2000). The levels of saponins in most common feed ingredients, including grain legumes, are rather low (Jezierny et al., 2010). Faba beans contain the two thermo-stable glycosides V + C. The use of faba beans in diets for non-ruminants is restricted mainly due to these glycosides (Crépon et al., 2010; Jezierny et al., 2010). Unlike the tannins located in the testa (Helsper et al., 1993), V + C located in the cotyledons are thermostable and therefore method to remove them have not been found (Dvořák et al., 2006; Vilariño et al., 2009; Crépon et al., 2010). V + C have a negative effect on production performance, especially of laying hens (Crépon et al., 2010; Jezierny et al., 2010). To my knowledge, there is a lack of study where the negative effects of V + C are more intensively studied.

Since the most modern varieties of *L. angustifolius* contain low concentrations of lectins and trypsin inhibitors (Pettersson, 2000) and alkaloids (Pettersson, 2000; Steenfeldt et al., 2003), the main problem in using lupins is that the hull of lupin seeds contain a high amount of fiber that consists of non-starch polysaccharides (NSP) (van Barneveld, 1999; Steenfeldt et al., 2003; Palander et al., 2006). In addition, the seed coats (hulls) of peas and faba beans contain NSP (Gatel, 1994; Castell et al., 1996). It is believed that NSP affects feed intake and digestibility (Carré et al., 1985) because no endogenous NSP-degrading enzymes are present in the avian intestinal system (Steenfeldt et al., 2003). Exogenous enzymes are needed to hydrolyze these highly branched substituents on the backbone (Igbasan and Guenter 1996; Cowieson et al., 2003). Therefore, there has been an interest in investigating whether exogenous enzymes can improve the nutritive value of legumes (Cowieson et al., 2003; Steenfeldt et al., 2003; Sahraei and Ghazi, 2012).

High levels of unprocessed peas of 250 – 500 g/kg in a laying hen diet have been shown to support good production (Castanon and Perez-Lanzac 1990; Ivusic et al., 1994; Perez-Maldonado et al., 1999; Fru-Nji et al., 2007). For faba beans, the acceptable levels have been lower than those of

peas. Crépon et al. (2010) reviewed a large number of publications and summarized that the use of faba beans with a high V + C content cannot exceed 70 g/kg of the laying hen diet, but it is possible to include faba beans at up to 200 g/kg if the cultivar used has a low V + C content. The many reports on using any of the *Lupinus* species in poultry diets note no deficiencies in performance, even with amounts up to 400 g/kg (Pettersen, 2000). However, most poultry farmers in Australia use a maximum of 200 g/kg *L. angustifolius* meal in laying hen diets because of the effect of the lupin NSP on digesta viscosity and the moisture content of excreta (Pettersen, 2000).

High levels of unprocessed peas at 300 – 480 g/kg in a broiler diet have been demonstrated to support good production (Farrell et al., 1999; Laudadio and Tufarelli 2010a; Dotas et al., 2014). In previous studies, 200 – 250 g/kg of faba beans were used in broiler diets without harmful effects on production performance (Farrell et al., 1999; Crépon et al., 2010; Gous, 2011). In contrast, *L. angustifolius* meal can be used in broiler diets at inclusion rates of at most 100 g/kg (Pettersen, 2000).

There is an interest in decreasing the use of imported SBM and in investigating alternative protein ingredients suitable for poultry diets. The optimal amounts of pea and faba bean inclusions determined in the previous studies varied. This may be due to genetic variation among varieties and hence to differences in their nutritive value and ANF contents. There is a strong interest in evaluating the nutritive values of locally produced and currently available grain legume cultivars for poultry and in determining their appropriate inclusion levels in the diets of laying hens and broilers. From consumers point of view there is also interest to study if pea and faba inclusions have any effect on the organoleptic quality test of meat.

2 AIMS OF THE STUDY

The main aim of this thesis was to increase knowledge of the protein quality and nutritive value of Finnish grain legumes in poultry diets. The primary goal was to improve domestic self-sufficiency in supplementary protein in poultry diets and decrease the dependence on imported SBM. The experiments were conducted to evaluate the following:

1. To find out appropriate inclusion levels of locally produced pea and faba bean seeds in diets of laying hens and broiler chickens while ensuring production performance and egg quality equal to those of birds fed diets based on cereals and SBM.
2. To determine the AME value and the digestibility of protein and AAs of pea, faba bean, lupin seeds, and broiler diets including faba beans.
3. To study the effects of dietary pea inclusion and the supplementation of an enzyme cocktail of xylanase (XLS), amylase (AMS), and protease (PRT) on broiler performance, intestinal viscosity, and the organoleptic quality of breast meat.
4. To determine the tannin and V + C contents of the most cultivated Finnish faba bean cultivar (cv. Kontu).

The main hypothesis tested in this research were:

1. Grain legumes can replace partly SBM in poultry diets. However, they contain ANFs that limit their use in poultry diets.
2. The energy values of grain legumes for poultry are good and their AAs are well digested.
3. Exogenous enzymes can improve nutrition value of diets containing grain legumes as shown by improved production performance of birds.

3 MATERIALS AND METHODS

The experiments (Expts) I – V (Studies I – V) were conducted at MTT Agrifood Research Finland in Jokioinen (Natural Resources Institute Finland (Luke) since 2015). The experimental procedures, chemical analyses, formulas used in calculation and statistical analysis were reported in detail in the original publications (I – V). A brief outline of the main design, analysis, and the measured parameters of each experiment is presented in this section. All the studies were approved by the Local or National Ethical Committee for Animal Experiments.

3.1 ANIMALS AND EXPERIMENTAL DESIGNS

The number of birds used and the main design of Expts I – V are shown in Table 1. The replicates were randomly assigned to feeding treatments. The experiments had a completely randomized blocking design.

Table 1. The design, aims, and measurements of experiments in Expts I – V.

Expt	Birds	Number of		Number of birds/		Aims	Measurements
		Treatment	Treatment	Replicate	Replicate		
I	576 Leghorn laying hens (Lohmann Selected Leghorn, LSL Classic)	4	8	18		The effects of dietary pea inclusion on performance and egg quality; To find an appropriate inclusion level of peas in the diet for layers	Performance and egg quality
II	560 Leghorn laying hens (Lohmann Selected Leghorn, LSL Classic)	5	7	16		The effects of dietary faba bean inclusion on performance and egg quality; To find an appropriate inclusion level of faba beans in the diet for layers	Performance and egg quality
III	3 000 508-Ross broilers	8	6	62/64		The effects of dietary pea inclusion together with the supplementation of a combination of xylanase (XLS), amylase (AMS), and protease (PRT) on broiler performance, intestinal viscosity, and the organoleptic quality of meat; To find an appropriate inclusion level of peas in the broiler diet	Performance, intestinal viscosity, and the organoleptic quality of meat
IV	196 508-Ross broilers	4	7	7 until d 24 / 4 after d 24		The effects of dietary faba bean inclusion on broiler performance, diet digestibility, and the nitrogen-corrected AME (AME _N) value of diet; To find an appropriate inclusion level of faba beans in the broiler diet	The AME _N value, total tract digestibility, and the coefficient of apparent ileal digestibility (CAID) of the nutrients
V	144 508-Ross broilers	6	6	4		The digestibility and AME value of two pea cultivars, two faba bean cultivars, and one lupin cultivar	The AME value and the CAID of the nutrients

3.2 HOUSING AND MANAGEMENT

Exp I was conducted in conventional cages (660 cm² total cage area offered per hen) and Expt II was conducted in enriched cages (750 cm² of total cage area per hen). Each photoperiod lasted 14.5 hours (I – II). The temperature in the hen house was kept at 20 °C (I – II).

In Expt III, the broilers were reared in floor pens (2m × 2m) with peat litter that each had approximately 60 broilers. The birds were sexed, and females and males were reared together. There was an equal number of females and males in each pen. In Expts IV and V, the broilers were reared in battery wire cages. At the beginning of Expt IV there were seven broilers and after 24 d of age four broilers per each cage. There were four broilers in each cage in Expt V. The temperature, light, relative humidity, and ventilation in the broiler barn were controlled according to Ross broiler breeder instructions (III – V) (Aviagen, 2014).

In Expts I and II, a chain feeder ran once a day to provide feed to the birds. In Expt III, feed was offered by metal feeders designed for this purpose. In Expts IV and V, feed was offered in the feed zone on the edge of the cages. Water was offered from nipple drinker lines. Feed and water were available *ad libitum* with the exception of the 24 hours of fasting in Expts IV and V.

3.3 DIETS

The dietary treatments used are shown in detail in Table 2. The main feed ingredients were sampled for chemical analysis before preparation of the experimental diets. The diet formulation was based on the analyzed chemical composition of the main ingredients and the table values for the other feed ingredients published in the Finnish Feed Tables and Nutrient Requirements (Luke, 2015). The diets were formulated to meet the nutrient requirements of LSL Classic hens (I, II) (Lohmann, 2010) or Ross broilers (III, IV) (Aviagen, 2014). In Expt V, the basal diet in was formulated to contain sufficient amounts of nutrients for broilers according to the Finnish Feed Tables and Feeding Recommendations (Luke, 2015). The nutrient contents of the diets were equalized in terms of energy (I – IV), protein (I, II, IV), AAs (I – IV), and the ratio of calcium to phosphorus (I – IV). The energy contents of the diets were equalized with rapeseed oil. The energy values (MJ AME per kg) were based on the feed values of feed ingredients published in the Finnish Feed Tables and Nutrient Requirements (Luke, 2015). The diets were formulated on a total AA basis, not on a digestible AA basis.

All the feed ingredients were coarsely ground in a roller mill (Gehl Company, West Bend, Wisconsin, USA), but the particle size was not determined. The feeds were mixed and cold-pelleted (Amandus Kahl Laborpresse 1175, Germany), except in Expt II, in which the feeds were mixed and steam-pelleted (Kahl 33-50, Amandus Kahl GmbH and Co. KG, Hamburg, Germany). In Expt II, both expander-processed and unprocessed faba beans were used.

Table 2. Experimental diets in Expts I – V.

Expt	Periods	Number of treatments	Treatments	Details
I	Phase I	4		
	21 – 41 wk of age			
	Phase II	4	Unprocessed pea seeds were included at 0 (control), 100, 200, and 300 g/kg of feed	Diets were equal in nutrients within feeding phases
	41 – 57 wk of age			
	Phase III	4		
II	57 – 73 wk of age			
	Phase I	5		
	39 – 59 wk of age		Control (diet without faba beans) + both unprocessed and expanded-processed faba bean seeds in proportions of 50 and 100 g/kg of feed	Diets were equal in nutrients within feeding phases
	Phase II	5		
	59 – 79 wk of age			
III	Starter diet	8	Four diets without (a) and 4 diets with (b) the addition of xylanase (XLS), amylase (AMS), and protease (PRT) (all 8 diets without pea inclusion)	Diets were equal in nutrients
	d 1 – d 9			
	Grower diet	8	Unprocessed pea seeds were included at 0 (control), 150, 300, and 450 g/kg of feed without (a) or with (b) addition of XLS, AMS, and PRT	The crude protein content was formulated to decrease with increasing dietary pea inclusion
	d 9 – d 38			
IV	Starter diet	1	Wheat and soybean meal (SBM) based control feed	
	d 1 – d 6			
	Grower diet	4	Unprocessed faba bean seeds were included at 0 (control), 80, 160, and 240 g/kg of feed	The diets were equal in nutrients; titanium oxide was used as an indigestible marker
	d 6 – d 32			
V	d 1 – d 20	1	Commercial starter feed based on wheat and SBM	
	d 20 – d 24	6	Solely basal diet + 5 diets consisted of 500 g of basal diet with 500 g of legume grain (two peas, two faba beans and one lupin, respectively) per kg. Diets were cold pelleted.	Chromic oxide was used as an indigestible marker.

3.4 GRAIN LEGUMES STUDIED

The grain legume seeds studied were from the two garden pea (*Pisum sativum* L subsp. *hortense*) cultivars cv. Karita (I, III, V) and cv. Sohvi (V), the two faba bean (*Vicia faba* L) cultivars cv. Ukko (V) and cv. Kontu (II, IV, V), and the lupin (*Lupinus angustifolius*) cultivar cv. Pershatsvet (V). The legume cultivars were the white-flowered leafed green smooth spring pea cv. Sohvi, the white-flowered semi-leafless green smooth spring pea cv. Karita, the coloured-flowered faba bean cultivars Ukko and Kontu, and the narrow-leafed lupin (blue lupin) cv. Pershatsvet. All these cultivars can be cultivated in the climatic conditions of southern Finland (dependent on cultivar in the growing zones I, II and III), but their harvesting as seeds is challenging in Northern Finland due to difficult growing conditions and a short season. The semi-leafless pea varieties are less susceptible to lodging than conventional leafed varieties and are favorable in monocultures (Uzun et al., 2004) and from that reason commonly used in Finland. Cultivar Karita is dominating cultivar in Finland, and was from that reason selected to include into poultry diets. Even though, it is well known that white-flowered faba beans are free of tannins and from that reason superior compared with coloured-flowered cultivars (flowers display large spots on the wings) when incorporated into poultry diets (Crépon et al., 2010), in the present study coloured-flowered varieties cv. Kontu and cv. Ukko were chosen to study. White-flowered varieties are not adapted to Finnish climate and cv. Kontu was only commercial variety at the time of experiments done.

3.5 CHEMICAL ANALYSIS AND DATA COLLECTION

Feed samples were taken from every batch made and then pooled (I, II, III). There was only one batch of each tested grain legumes in digestibility studies (IV, V). The feed samples were passed through a hammer mill fitted with a 1-mm mesh prior to analysis. The V + C content of the faba bean seeds (cv. Kontu) was determined in Expts II and IV and the tannin content of the faba bean seeds (cv. Kontu) was determined in Expt IV.

Egg weight and number were recorded daily, and the mean production variables were calculated for each 4-week period (I, II). The feed intake was recorded throughout and calculated for each 4-week period. The Finnish Food Safety Authority diagnosed the cause of death and performed autopsies on one hen per replicate euthanized after the Expt II.

In Expt III, the birds were weighed at the ages of 1 d and 9 d and at the end of the experiment (37 d). In Expt IV, the broilers were weighed at the ages of 1 d and 6 d and at the end of the experiment (32 d). The feed

intake was recorded throughout and calculated for each period. In Expt III, the carcass weight of each pen was measured at a commercial slaughterhouse. The organoleptic quality (taste, tenderness, and juiciness) of breast meat samples after roasting was performed by a panel of experts. The ileal digesta was quantitatively collected from the distal part of the ileum for viscosity determination.

In Expts IV and V, the experimental period consisted of an adaptation period of 26 d, an excreta collecting period of 4 d. Due to practical reason there was 3 days gap after excreta collection followed by ileal digestibility assay, which consisted 1 day of fasting (24 h), 4 hours free access to feed (birds were allowed to eat *ad libitum*) and slaughtering of birds. Feed consumption was measured during the excreta collection period and on the slaughtering day. The ileal digesta was quantitatively collected from the entire ileum or the distal part of the ileum to determine the coefficients of apparent ileal digestibility (CAID).

The mortality was recorded daily (I – IV). The cumulative mortality rates were calculated after completion of the experiments.

3.6 CALCULATION AND STATISTICAL ANALYSIS

The AME values, and the CAIDs of nutrients of grain legumes (V), and the nitrogen retention, nitrogen-corrected apparent metabolizable energy (AME_N) values, and the CAIDs of nutrients of faba bean diets were calculated (IV). The AME and the CAIDs of the nutrients of grain legumes were calculated using the difference method (V).

The experimental data were tested for normality with the Shapiro-Wilk test. The data was subjected to ANOVA using the GLM procedure of SAS (SAS Institute Inc., Cary, NC, USA) with a replicate as the experimental unit. Differences were considered to be significant at $P \leq 0.05$, and $P < 0.10$ tended to be significant.

In Expt I, the treatment effects were separated into two polynomial contrasts (the linear and quadratic effect of dietary pea inclusion). Expt II had a 2×2 factorial design with two dietary faba bean inclusions (50 or 100 g faba beans per kg diet) and two faba bean types (unprocessed vs. expander processed). In addition, there was a control treatment without faba bean inclusion in the experiment. The treatment effects were separated into four orthogonal contrasts (presented in paper II). Expt III had a 4×2 factorial design with four increasing dietary pea inclusions (0, 150, 300, or 450 g peas per kg diet) and two type of diets either with or without enzyme additions of XLS, AMS, and PRT. The treatment effects were separated into six orthogonal contrasts

(presented in paper III). Because the data of organoleptic quality of breast meat in Expt III was not normally distributed, it was analysed by non-parametric Kruskal-Wallis test.

In Expt IV, the treatment effects were separated into two polynomial contrasts (the linear and quadratic effect of dietary faba bean inclusion). In Expt V, all possible significant pairwise differences between the test legumes were detected with the Tukey Kramer test.

4 RESULTS AND DISCUSSION

The main aim of this thesis was to increase knowledge of the nutritive value of locally produced grain legumes in poultry diets. To achieve this, the five studies (I – V) were performed that are presented in detail in the section of materials and methods. However, narrow-leaved lupin (blue lupin) (*Lupinus angustifolius*) was studied only in respect of AME and the CAIDs of nutrients in broiler diets (V), and it is not extensively discussed otherwise.

4.1 CHEMICAL COMPOSITION OF GRAIN LEGUMES

4.1.1 Protein and amino acid content

Table 3 presents the crude protein (CP) (I – V) and AA contents (V) of grain legumes and SBM (Luke, 2015). The CP content of legumes (g/kg dry matter (DM) basis) ranged from 199 (pea cv. Sohvi) to 318 (faba bean cv. Kontu) (I – V). Considering the large variation in legume protein content, the values in the current study were at the same level as those reported in the literature (Savage and Deon 1989; Gatel and Grosjean 1990; Petterson et al., 2000; Crépon et al., 2010; Nalle et al., 2011a; Kaczmarek et al., 2014). The protein content of grain legumes is known to vary greatly between cultivars, ranging from 156 to 346 g/kg DM for pea (Savage and Deon 1989; Gatel and Grosjean 1990), from 270 to 320 g/kg DM for faba bean (Crépon et al., 2010) and from 223 to 400 g/kg DM for lupin (*Lupinus angustifolius*) (Petterson et al., 2000; Palander et al., 2006, Nalle et al., 2011a; Kaczmarek et al., 2014).

Protein contents in peas and lupin was approximately 40 %-units of the protein content from SBM. Faba beans contained more protein than peas and lupin, but approximately 60 %-units of the protein content from SBM. Because grain legumes contained less protein compared with SBM, their AA contents (g/kg DM) compared with SBM were lower as well.

In general, the CP content of garden peas is lower than that of ordinary field peas (*Pisum sativum* L subsp. *arvense*) (Rodrigues et al., 2012). However, there seems to be a lack of data comparing the feeding value of garden and field peas, and it is not always reported in detail which type of pea has been studied. However, Bastianelli et al. (1998), studied different pea lines and summarized that white-flowered garden peas used for feed and food are round and have similar composition in terms of protein, starch and fibre contents; they are tannin-free and have variable trypsin inhibitor activity. Coloured-flower field peas have also a round shape but differ from the garden peas principally by tannins and also by lower starch, higher protein, higher fibre

contents. Wrinkled peas differ from the garden peas by lower starch, higher protein, fibre, and lipid contents and their starch is characterized by a higher amylose/amylopectine ratio. An unexpected result of Expt V was that the CP content of the semi-leafless pea cv. Karita was higher than that of the leafed pea cv. Sohvi. Usually the CP content of semi-leafless peas is lower than that of leafed peas (Niskanen, 2000).

The AA content of all grain legume types studied in the present study was determined in Expt V. Arginine was the most abundant essential amino acid (EAA) (≥ 7.9 g/100 g CP), whereas glutamine was found to be the most abundant non-essential amino acid (NEAA) (≥ 15.0 g/ 100g CP). Methionine was the most limiting EAA (≤ 0.9 g/ 100g CP), whereas semi-essential cysteine was the most limiting NEAA (≤ 1.7 g/ 100g CP). The lysine and methionine+cysteine contents of legumes (g/kg DM basis) varied between 10.5 (blue lupin cv. Pershatsvet) and 19.0 (faba bean cv. Kontu) and between 5.0 (pea cv. Sohvi) and 5.7 (faba bean cv. Kontu). The threonine content of legumes varied between 6.8 (pea cv. Sohvi) and 11.4 (faba bean cv. Kontu), respectively.

Compared to the AA content of SBM protein, the proteins of pea and faba bean cultivars were equally good sources of lysine, whereas lupin protein was less abundant source of lysine. Compared to SBM protein, the protein of all grain legume cultivars were deficient in methionine. This is due to the AA composition of the main storage protein globulins, whose sulfur AA content is lower than in albumins typical for SBM (Gatel, 1994). However, grain legume proteins had as much cysteine as SBM protein. The proteins of peas and, the faba bean cv. Kontu, and lupin were deficient in threonine, as previously shown for lupin by Wiseman and Cole (1988). Grain legumes are usually also deficient in tryptophan (Gatel, 1994).

The AA contents of peas (V) were in accordance with previous reports (Gatel, 1994; Nalle et al., 2011b; Masey O'Neill et al., 2012) with the exception of the higher methionine content reported by Nalle et al. (2011b). However, considering the range of AA contents of peas reported by Castell et al. (1996), the amounts of all AAs in peas and especially in the cv. Karita were high. The AA content of faba beans was in line with Gatel (1994) but higher than reported by Gous (2010), Nalle et al. (2010), and Masey O'Neill et al. (2012), with the exception of the lower methionine and cysteine contents especially in the faba bean cv. Kontu. The AA contents of lupin (*L. angustifolius*) were lower than reported by Petterson et al. (2000) and Nalle et al. (2011a) for Australian and New Zealand lupins (*L. angustifolius*) but were in accordance with the contents presented by Sujak et al. (2006) for European lupins (*L. angustifolius*).

The AA content of the legume batches studied (V) was in accordance with previous reports on the same Finnish legume varieties (Partanen et al., 2001; Palander et al., 2006). Pea protein contained approximately 13

%-units more lysine, methionine, cysteine, and threonine than faba bean and lupin proteins. However, there were two exceptions: the highest methionine content was in faba bean cv. Ukko protein, and the highest cysteine content was in lupin protein. The reason for these unexpected results may be related to the AA analysis used. Pea protein also contained more phenylalanine, alanine, and tyrosine than faba bean and lupin proteins.

Table 3. Chemical composition of soybean meal (SBM) and grain legumes (I – V).

	SBM		Peas		Faba beans		Lupin	
		cv. Sohvi ¹	cv. Karita ²	cv. Kontu ³	cv. Utko ¹	cv. Pershatsvet ¹		
CP, g/kg DM	447 (V) – 547 (III)	199	217 (V) – 236 (II)	274 (III) – 318 (V)	296	223		
Ether extract, g/kg DM	12 (IV) – 46 (V)	27	14 (II) – 27 (V)	13 (III) – 26 (V)	29	81		
Crude fibre, g/kg DM	41 (I) – 67 (V)	75	49 (V) – 55 (II)	77 (III) – 94 (II)	116	199		
EAA, g/100 g CP								
Arginine	7.7 ⁵	7.91	7.98	8.96	9.15	8.51		
Histidine	2.9 ⁵	2.60	2.94	2.61	2.73	2.60		
Isoleucine	4.7 ⁵	4.33	4.25	3.67	3.96	4.62		
Leucine	8.3 ⁵	6.58	6.70	6.57	7.28	5.83		
Lysine	6.2 ⁵	7.26	7.20	5.97	6.14	4.70		
Methionine	1.4 ⁵	0.84	0.72	0.52	0.86	0.67		
Phenylalanine	5.4 ⁵	4.76	4.83	3.98	4.25	3.47		
Threonine	4.0 ⁵	3.42	3.40	2.96	3.85	2.90		
Valine	4.9 ⁵	3.75	3.97	3.41	5.51	3.76		
NEAA, g/100 g CP								
Alanine	4.6 ⁵	4.51	4.59	3.97	4.20	3.51		
Asparagine	12.4 ⁵	10.1	10.5	9.28	30.8	8.20		
Cysteine	1.3 ⁵	1.67	1.54	1.26	1.39	1.72		
Glutamine	18.9 ⁵	16.7	17.34	15.7	15.0	19.2		
Glycine	4.3 ⁵	4.45	4.33	3.95	4.22	3.83		
Proline	6.1 ⁵	4.08	5.00	4.27	4.08	4.55		
Serine	5.2 ⁵	4.64	4.75	4.28	4.88	4.31		
Tyrosine	3.8 ⁵	3.41	3.37	3.16	3.36	3.11		

EAA = essential amino acid, NEAA = non-essential amino acid, nd = not determined, CP = crude protein

¹ V; ² I, III and V; ³ II, IV and V; ⁴ II; ⁵ (> 500 g/kg CP) (Luke, 2015)

4.1.2 Anti-nutritional factors

The V + C content of unprocessed faba beans (cv. Kontu) seeds was 10.6 (II) and 9.2 g/kg DM (III) (respectively), whereas it was 8.9 g/kg DM (II) for expander-processed faba beans. Considering the small difference between 10.6 and 8.9 g/kg and considering that this result was not statistically tested, one cannot say that expander-processing reduces the V + C content of faba beans (II). The V + C contents of unprocessed faba bean seeds agree with the results of Duc et al. (1999), who reported that the mean V + C content for the high V + C genotype ranged from 6 to 14 g/kg DM. Jezierny et al. (2010) reported a V + C content of 0.3 g/kg DM for the low V + C genotype.

Faba bean (cv. Kontu) seeds had a high tannin content of 13.7 g/kg DM (III). The tannin content of tannin containing cultivars (coloured-flower cultivars) typically ranges from 5 to 10 g/kg DM (Duc et al., 1999). Crépon et al. (2010) reviewed that the mean tannin content of tannin-free cultivars (white flowers) is 0.1 g/kg DM (Bond and Duc, 1993). To my knowledge, no research studies have previously been conducted on the V + C and tannin content of the faba bean cv. Kontu and nor of the the cv. Ukko. Since the cv. Ukko has colored flowers (displays large spots on the wings), it is likely to be a tannin containing variety.

Unlike the tannins located in the testa (Helsper et al. 1993), V + C located in the cotyledons are thermostable and therefore cannot be easily removed through technological processes, not even at high temperatures (Dvořák et al., 2006; Vilariño et al., 2009; Crépon et al., 2010). In the study of Dvořák et al. (2006), different methods of treatment were tested – including exposure to over 100 °C, to organic acids, and to substrate maturation (which exposes seeds to high temperature) along with subsequent drying – without any significant effects on the V + C content. For these reasons, expander-processing had no effect on the V + C content of faba beans.

4.2 IDEAL PROTEIN OF LEGUMES

The figure 1 presents the AA composition of the selected AAs in legumes (V, Pérez et al., 1993) corresponding to Illinois Ideal Chick Protein (IICP) (Emmert and Baker, 1997). The IICP is a system where the requirements of main AAs, which may be limiting in broiler feeds, are calculated and then lysine is used as the reference AA to which ratios are set for other AAs. In this system, true ileal AA digestibility was used (Baker and Han, 1994). However, in figure 1 values presented for legumes are based on the CAIDs of AAs (V). The values based on experiment V and Pérez et

al., (1993) can still be compared to IICP though in case of threonine values can be slightly underestimated due to threonine secreted in endogenous protein. Endogenous secretions in chickens are known to contain relatively high concentrations of threonine (Siriwan et al., 1994). Average values were used in calculation of AAs ratios for pea and faba bean (V). For SBM they were calculated from AA contents and the CAIDs of AAs presented by Pérez et al. (2010).

The methionine ratio was better and threonine ratio was slightly better in SBM than in grain legumes. Lupin had better cysteine and methionine + cysteine ratios compared to that of SBM. However, methionine, cysteine, methionine + cysteine, and threonine ratios in any legume (including SBM) did not respond to the ratio required. The valine ratio of SBM and pea was lower than required, while in faba beans and especially in lupin it responded to the ratio required. The arginine ratio of SBM and pea responded well to the ratio required, whereas in faba beans and lupin it was remarkably higher than required. It can be confirmed, that among grain legume proteins lupin protein responded best to the IICP. However, as SBM had the best ratios of methionine and threonine, which are EAA, SBM meets better IICP than grain legumes.

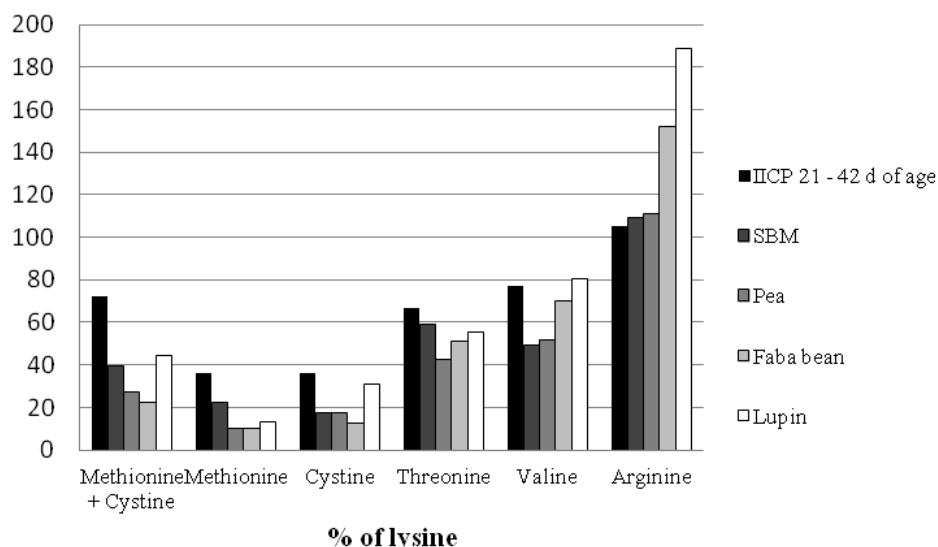


Figure 1. Ratios of amino acids in grain legumes (V) and in soybean meal (Pérez et al. 1993) corresponding to Illinois Ideal Chick Protein, IICP (Emmert and Baker 1997).

4.3 EFFECTS OF PEAS ON LAYING HEN PERFORMANCE

4.3.1 Production parameters

Dietary pea inclusion of up to 300 g/kg had no effects ($P > 0.05$) on the egg production (%), egg weight (g), and egg mass production (production, g per hen per d) (I). Table 4 presents the optimum inclusion levels of peas in laying hen diets presented in the literature. The egg production variables were comparable to those in earlier studies with high inclusion levels (250 – 500 g/kg) of peas (Ivusic et al., 1994; Perez-Maldonado et al., 1999; Fru-Nji et al., 2007). It can be confirmed, that the appropriate pea inclusion in laying hen diets appears to be at least 300 g/kg.

4.3.2 Feed intake and feed conversion ratio

Dietary pea inclusion of up to 300 g/kg had no effects on the feed intake and FCR ($P > 0.05$), in line with the results by Ivusic et al. (1994) using pea inclusion of up to 590 g/kg (I). Fru-Nji et al. (2007) found increased feed intake when peas were included at a level of 500, whereas the feed conversion ratio (FCR) was unaffected by pea inclusion.

4.3.3 Egg quality

The values of the egg quality variables studied – specific weight, Haugh unit, and shell strength – were similar in all the feeding treatments ($P > 0.05$) (I). Results for the egg quality variables agree with the results of Fru-Nji et al. (2007), who found no significant differences in the egg quality variables in diets with up to 500 g/kg of peas. Anderson (1979) found no significant differences in egg quality variables (albumen quality, yolk color, and chemical composition) when 300 g/kg of peas were included in the diet, but showed that pea inclusion had an adverse effect on shell quality. Ivusic et al. (1994) reported that feeding diets with 590 g peas per kg of feed resulted in eggs with thinner shells.

4.4 EFFECTS OF FABA BEANS ON LAYING HEN PERFORMANCE

4.4.1 Production parameters

Expander-processing of faba beans did not have an effect ($P > 0.05$) on the production performance of the hens (II). The only significant effect of faba bean supplementation was decreased egg weight (g) ($P \leq 0.05$) compared to the control diet. Table 4 presents the optimum inclusion levels of faba beans in laying hen diets and the effects of faba bean inclusion on performance presented in the literature. Decreased egg weight was found using less faba beans than in previous studies, in which faba bean inclusion ranged from 100 to 150 g/kg (Davidson et al., 1973; Robblee et al., 1977; Campbell et al., 1980; Olabora et al., 1981). Egg mass production (production, g per hen per d) decreased ($P \leq 0.05$) when the faba bean inclusion level increased from 50 to 100 g/kg of feed. This supports the findings of Halle et al. (2005) and Fru-Nji et al. (2007), which showed reduction in egg mass production with increased dietary faba inclusion. The reductions in egg weight and daily egg mass production were most likely due to V + C.

Muduuli et al. (1981) demonstrated that vicine consumption reduced egg weight and increased erythrocyte hemolysis (the breakdown of red blood cells). According to Muduuli et al. (1981), vicine can act in the following three ways: by reducing the amount of precursor material available to the granulosa cells, by damaging granulosa cells and hence their activity, or by destroying the ovum. Additional crystalline methionine is beneficial to prevent egg weight loss (Davidson et al., 1973; Campbell et al., 1980) and egg mass production (Fru-Nji et al. 2007) when faba beans are used in layer diets.

In Expt II, methionine was supplied to achieve the nutrient requirements of the hens (Lohmann, 2010). The differences in CP content of the experimental diets were small. In addition, there were no differences in the detected feed consumption rates, so these do not seem to have had an effect on egg weight or egg production. These considerations support the contention that the reductions in egg weight and daily egg mass production were most likely due to V + C. In other respect, the use of faba beans could be way to prevent egg weight increase with older hens. However, it should be further studied does decrease egg weight always due to damaged granulosa cells, which impair health and likely as well welfare of birds.

It seems that the optimal inclusion level for the faba bean cv. Kontu in laying hen diets is 50 g/kg (II), which is in line with Crépon et al. (2010), who summarised that the use of faba beans with a high V + C content cannot exceed 70 g/kg of the diet. However, it is possible to include faba beans at up to 200 g/kg if the cultivar used has a low V + C content (Crépon et al., 2010). Campbell et al. (1980) summarized that when diets adequately supplemented with methionine were used, egg production rates of hens fed faba beans were similar to those of controls except for high dietary levels of faba beans (in excess of 257 g/kg) where a decrease was registered.

4.4.2 Feed intake and feed conversion ratio

Faba bean inclusion had no effect on feed consumption ($P > 0.05$), which is in agreement with Robblee et al. (1977), Muduuli et al. (1981), and Fru-Nji et al. (2007) (II). The FCR increased when dietary faba bean inclusion increased from 50 g/kg to 100 g/kg ($P \leq 0.05$), in line with Davidson et al. (1973), Robblee et al. (1977), and Fru-Nji et al. (2007), who used faba bean inclusion ranging from 150 to 200 g/kg. The FCR increased because of decreased production and the unaffected feed intake due to faba bean inclusion. This may be partially explained by the harmful effect of ANF on diet digestibility, as shown by Fru-Nji et al. (2007). In agreement with Fru-Nji et al. (2007), in the present study faba beans were included in their raw form, leaving their maximum ANF effect, unlike SBM that has undergone physical processing, including thermal treatment, which is well known to improve legume protein digestibility (Fru-Nji et al., 2007).

4.4.3 Egg quality

Faba bean inclusion up to 100 g/kg had no effect ($P > 0.05$) on the egg quality variables studied (specific weight, Haugh unit, shell strength, shell thickness) (II). This agrees with the results of Fru-Nji et al. (2007) (shell strength), Laudadio and Tufarelli (2010b) (Haugh unit and shell thickness), and Robblee et al. (1977) (specific weight), who studied diets with up to 400 g/kg of faba beans. Laudadio and Tufarelli (2010b) studied dehulled micronized faba beans, whereas Robblee et al. (1977) and Fru-Nji et al. (2007) studied unprocessed faba beans. In contrast to the current study, the Haugh unit values in the study by Robblee et al. (1977) increased as the amount of faba beans in laying hen diets increased from 0 to 300 g/kg. The reason for this is most likely the higher faba bean inclusion level used by Robblee et al. (1977) compared with the levels studied (II). In the studies by Fru-Nji et al. (2007) and Laudadio and Tufarelli (2010b), the albumin fraction (used in Haugh unit measurement) was found to increase with faba bean inclusion. In the study by Fru-Nji et al. (2007),

an increase in the albumin fraction compensated a reduction in yolk fraction. Fru-Nji et al. (2007) found that faba beans seem to increase the viscosity of the albumen. They theorized that since albumen is formed in the magnum followed by water being added to it in the uterus, the influence of faba bean could be at two possible sites of egg formation. Either in the magnum, where more concentrated albumen is produced or in the uterus, where less water is probably added.

Table 4. Optimum inclusion levels of unprocessed peas and faba beans in laying hen diets¹ presented in the literature.

Reference	Type	Birds used	Optimum inclusion level	Impact on performance (only if reported)
Peas				
Castanon and Perez-Lanzac (1990)	Cull peas	Laying Leghorn hens	333 g/kg	
Ivusic et al. (1994)	Yellow pea cv. Miranda	Dekalb XL Single Comb	445 g/kg	
Perez-Maldonado et al. (1999)	Field pea cv. Glenroy	White Leghorn (SCWL) SIRO-CB pullets	250 g/kg	
Halle et al. (2005)	White-flowered pea	Laying leghorn hens (LSL Classic)	400 g/kg	
Fru-Nji et al. (2007)	Field pea	Half were LSL Classic and the other half Lohmann	500 g/kg	
Faba beans				
Robblee et al. (1977)	Faba bean cv. Ackerperle	White Leghorn pullets	Not more than 100 g/kg	Egg weight loss
Campbell et al. (1980)	not reported	Single Comb White Leghorn (SCWL) hens	Less than 250 g/kg	Reduced egg production rate and egg weight
Castanon and Perez-Lanzac (1990)	not reported	SIRO-CB pullets	Less than 200 g/kg	Negative effect on daily egg production and egg weight
Perez-Maldonado et al. (1999)	Faba bean cv. Fiord	SIRO-CB pullets	Less than 250 g/kg	Negative effect on daily egg production and egg weight
Halle et al. (2005)	Faba bean cv. Gloria (white-flowered) and cv. Scirocco (coloured-flowered)	Laying leghorn hens (LSL Classic)	100 g/kg	Reduced egg mass production and reduced feed intake ²
Fru-Nji et al. (2007)	Faba bean: Trace of tannins: 0.2 g/kg, trace of V + C content: 8.8 g/kg	Half were LSL Classic and the other half Lohmann Brown	160 g/kg	Reduced egg production and the some egg quality traits

¹ supplemented with adequate levels of methionine and adjusted to meet the requirements of the laying hens

² Due to hen losses in the groups with 200 and 300 g/kg Scirocco and 300 g/kg Gloria

4.5 EFFECTS OF PEAS ON BROILER PERFORMANCE

4.5.1 Body weight, carcass weight, and body weight gain

Table 5 presents the optimum inclusion levels of peas in broiler diets presented in the literature. Body weight, carcass weight, and body weight gain changed ($P \leq 0.05$) with pea inclusion in a cubic manner during the grower period and the entire experiment (III). The growth performance of the broilers was comparable to that of birds in earlier studies, in which pea inclusions between 100 and 480 g/kg had no effect on body weight (Igbasan and Guenter 1996; Laudadio and Tufarelli 2010a; Dotas et al., 2014). Laudadio and Tufarelli (2010a) studied micronized dehulled peas, whereas Igbasan and Guenter (1996) and (Dotas et al., 2014) studied unprocessed peas. Numerical data suggests that pea inclusion at a level of 150 g/kg supports better body weight and body weight gain than in control diets and in diets that included higher amounts of peas. It is likely that this is due to diets with 150 g/kg of peas having higher AA contents than other diets. However, at least 450 g/kg of peas can be used in broiler diets because this did not have any negative affect compared to control diet.

4.5.2 Feed intake and feed conversion ratio

Pea inclusion had no effect on feed consumption ($P > 0.05$) (III), which is line with the results of Igbasan and Guenter (1996), Laudadio and Tufarelli (2010a), and Dotas et al. (2014). The FCR of the birds fed unsupplemented diets improved with pea inclusion during the grower period and the entire experiment. However, the improvement in FCR was very slight compared to the control group, especially when the inclusion level was 450 g/kg. Pea inclusions of 150 and 300 g/kg had no effect on FCR, and pea inclusion of 450 g/kg impaired FCR of birds on XLS, AMS, and PRT supplemented diets. (The interaction of the linear effect of dietary pea inclusion and enzyme supplementation was significant ($P_L \times E$), $P \leq 0.05$.) The findings for unsupplemented diets support previous studies in which green or light coloured pea seeds or pea meal improved FCR (Igbasan and Guenter, 1996). The impaired FCR of birds on XLS, AMS and PRT supplemented diets was in line with the results by Cowieson et al. (2003). However, as with growth performance, there was also a numerical improvement in the FCR of birds on a diet with 150 g/kg of peas compared with the FCR of birds on the control diet. In the studies by Dandanell Daveby et al. (1998), Laudadio and Tufarelli (2010a), and Dotas et al. (2014), pea inclusion had no effect on FCR.

4.5.3 Ileal viscosity

Increasing digesta viscosity results in sticky droppings and increased litter moisture content with negative effect of bird health (Francesch and Brufau 2004). Low ileal viscosity is priority in poultry industry, to avoid environmental and animal welfare problems as dermatitis and to reduce productivity losses as impaired FCR and growth (Francesch and Brufau 2004). Pea inclusion tended to decrease ($P \leq 0.10$) the intestinal viscosity of the birds in a linear manner. Decreased ileal viscosity was connected with decreased amounts of wheat in the diet (III). High levels of wheat increased viscosity in small intestinal digesta due to the presence of a soluble high molecular carbohydrate component (arabinoxylans) (Annison et al., 1993). Moreover, the lower bird intestinal viscosity caused by dietary pea inclusion indicated that the pea cultivar studied did not contain harmful levels of water-soluble polysaccharides, suggesting that the water-soluble polysaccharides of peas increase intestinal viscosity (Igbasan and Guenter 1996). The result of the intestinal viscosity tests support the findings by Farrell et al., (1999), who demonstrated in testing field peas, faba beans, chick peas, and sweet lupins that only lupins increased intestinal viscosity.

4.5.4 Organoleptic quality of breast meat

The present results in the organoleptic quality test of breast meat agree with the results of McNeill et al. (2004), which showed that the inclusion of field peas at up to 200 g/kg had no effect on the flavor of breast meat (III). To my knowledge, no research studies have previously been conducted on the effects of dietary pea inclusion on the tenderness and juiciness of breast meat.

4.5.5 Effects of pea and enzyme combination on broiler performance

The use of an enzyme cocktail improved the nutritive value of wheat-pea diets as evidenced by the improvements ($P \leq 0.05$) in performance of the broilers and by decreased intestinal viscosity ($P \leq 0.05$) (III). The improvements in production performance due to the addition of XLS, AMS and PRT were most likely associated with more intensive hydrolyzation of the starch and fibre carbohydrates of the wheat than the peas.

Igbasan and Guenter (1996) found that the addition of a combination of PRT and pectinase to diets including peas did not produce a response in bird performance, but the use of pectinase alone

increased weight gain. Brenes et al. (1993) found no improvement in the growth of birds on diets including peas but found improvements in the FCR when crude enzyme preparations (cell-wall-degrading multi-enzyme complexes) and α -AMS were added to diets containing peas. Moreover, Brenes et al. (1993) stated that the effect of enzyme supplementation appeared to be pea variety dependant and was only beneficial when added to diets with tannin containing peas. Cowieson et al. (2003) found that improvements in broiler performance through use of a combination of cellulose, XLS, and AMS were dependent on the pea cultivar used. However, it should be pointed out that the studies by Brenes et al., (1993), Igbasan and Guenter (1996), and Cowieson et al. (2003) investigated different combinations of exogenous enzymes, so the results of these studies are not fully comparable.

Even though the effects of XLS, AMS, and PRT could not be distinguished, I assume that the improvements in bird performance (III) were more likely due to AMS and XLS than PRT, as shown in the literature (Longstuff and Mc Nab, 1987; Brenes et al., 1993; Igbasan and Guenter 1996; Cowieson et al., 2003). Reduced intestinal viscosity due to enzyme supplementation supports this contention. Arabinoxylans have been shown to reduce chick performance and increase ileal viscosity (Annison, 1993). Cowieson et al. (2003) have previously suggested that the presence of XLS could be responsible for some of the improvements in weight gain and FCR through a reduction in the anti-nutritive effects associated with wheat arabinoxylans. Peas have studied to contain minimal amount of arabinoxylans (Bach-Knudsen, 1997; Glada, 1998). Whereas cellulosa is an important dietary fibre constituent of the cell walls of protein rich materials as pea and faba beans, which also contain relatively high levels of pectic polysaccharides (Bach-Knudsen, 1997). According to Longstuff and Mc Nab (1987), the complex polysaccharide mixture in peas is particularly degraded through cellulose and AMS, which indicates that the nutrient availability of peas may be improved by AMS. In addition, a reduction in luminal viscosity is associated with hydrolyzation of wheat NPS or pea starches (Cowieson et al., 2003). The results of the current study indicate that the addition of AMS reduced the anti-nutritive effects of the starch carbohydrates in wheat and peas whereas the addition of XLS led to breakdown of arabinoxylans, which are the main cell wall polysaccharides in wheat.

The major limitation of study (III) was that the composition of dietary fibre was not determined and starch content in feed ingredients used was not analyzed. These measurements would help us confirm that the positive effect of enzyme combination was due to degradation of backbone

substituent of wheat. In addition of that, enzyme cocktail chosen was not appropriate to improve utilization of peas.

4.6 EFFECTS OF FABA BEANS ON BROILER PERFORMANCE

4.6.1 Body weight and body weight gain

The birds were reared in wire cages, so the measured bird performance is not fully comparable to the performance achieved on farms (IV). Body weight and body weight gain were reduced ($P \leq 0.05$) in a linear manner by faba bean inclusion. However, the reduction in body weight was not clearly seen until faba bean inclusion was 240 g/kg. Table 5 presents the optimum inclusion levels of faba beans in broiler diets and the effects of faba bean inclusion on performance presented in the literature. Results (IV) agree with those of Rubio et al. (1990), which show that body weight decreased when 250 g/kg or more faba beans were used in the diet. Results (IV) also support the findings of Farrell et al. (1999) and Nalle et al. (2010), who found no difference in body weight gain between broilers on a control diet and broilers fed up to 200 g/kg faba beans.

4.6.2 Feed intake and feed conversion ratio

The feed intake of the broilers decreased ($P \leq 0.05$) and FCR improved ($P \leq 0.05$) in a linear manner with increasing faba bean inclusion (IV). The decreased feed consumption with faba bean inclusion was in agreement with Rubio et al. (1990). The tannins in faba beans have been shown to decrease feed intake (Ortiz et al., 1994; Helsper et al., 1996) and feed palatability (Berger et al., 2003). It is possible that the decreased feed consumption and further decreased body weight and body weight gain with faba bean inclusion were at least partly due to the poor palatability of faba beans with high tannin content. In contrast to present findings (IV), Farrell et al. (1999) and Nalle et al. (2010) observed no differences in feed intake between broilers on a control diet and others fed up to 200 g/kg faba beans in the diet. Farrell et al. (1999) did not report tannin content, and Nalle et al. (2010) studied cultivars with low tannin content. In contrast to current findings (IV), faba bean inclusion in previous studies had no effect on FCR (Nalle et al., 2010) or impaired it (Rubio et al., 1990).

The recommended faba bean inclusion level of 160 g/kg (IV) is in line with Farrell et al. (1999) (200 g/kg) and Nalle et al. (2010) (200 g/kg). However, Gous (2011) reported that faba beans are an

alternative protein source in feeds for broilers up to an inclusion level of 250 g/kg. A possibly smaller tannin and V + C contents in the study by Gous (2011) (not measured), may explain the difference in performance compared those found in this study (IV).

Table 5. Optimum inclusion levels of unprocessed peas and faba beans in broiler diets¹ presented in the literature.

Reference	Type	Birds used	Optimum inclusion level	Impact on performance (only if reported)
Peas				
Ighasan and (1996)	Guenter Smooth green (cv.), yellow (cv.) and brown (cv.) peas	Broiler chicks	200 g/kg for yellow and green peas and less than 200 g/kg for brown peas	Impaired weight gain and FCR
Farrell et al. (1999)	Field pea cv. Glenroy	Male broiler chicks	300 g/kg	
Dotas et al. (2014)	Field pea cv. Olympos	Ross 308 male chickens	480 g/kg	
Faba beans				
Rubio et al. (1990)	Faba bean var. Prothabon	Hubbard male chickens	Less than 250 g/kg	Decreased body weight
Helsper et al. (1996)				
Farrell et al. (1999)	Faba bean cv. Fiord		200 g/kg	
Nalle et al. (2010)	Low tannin faba beans cv. PGG Tic, cv. Spec Tic, cv. South Tic and cv. Broad	Ross 308 male chickens	200 g/kg	
Gous (2011)	Faba bean cv. Fiord	Broiler chicks	250 g/kg	

nr = not reported

¹ supplemented with adequate levels of methionine and adjusted to meet the requirements of the birds

4.7 BIRD MORTALITY

4.7.1 Effects of pea inclusion

Pea inclusion of up to 300 g/kg of the diet had no effect on laying hen mortality (I). This was in line with Ivusic et al. (1994), and Igbasan and Guenter (1997) reported no differences in mortality between the control diet and diets with pea inclusion. Pea inclusion of up to 450 g/kg of the diet had no effect on the mortality of broilers (III) in agreement with Farrell et al. (1999), Laudadio and Tufarelli (2010a), and Dotas et al., (2014) reported no differences in mortality between the control diets and diets with peas.

4.7.2 Effects of faba bean inclusion

Mortality tended to increase ($P \leq 0.10$) when 100 g/kg of faba beans were added to a laying hen diet (II). In the study of Robblee et al. (1977), a faba bean inclusion level of 200 g/kg feed had no adverse effect on the mortality of white Leghorn pullets, but a level of 300 g/kg feed increased the mortality rate. It is conceivable that the V + C content of the faba beans studied (cv. Kontu) may have been higher than that of the faba beans (cv. Ackerperle) used by Robblee et al. (1977), who did not report the V + C content of the cultivar they used. In the study of Fru-Nji et al. (2007), mortality rates were not reported, indicating that faba bean inclusion had no major effect on the mortality of the hens in their study (half were LSL and the other half Lohmann Brown). Faba bean inclusion also had no adverse effect on mortality in the studies of Campbell et al. (1980), Perez-Maldonado et al. (1999), and Laudadio and Tufarelli (2010b). Campbell et al. (1980) used SCWL laying hens, Hy-line, and at 250 g/kg of faba beans. Perez-Maldonado et al. (1999) used SIRO-CB pullets and 250 g/kg of the faba bean cv. Fiord. Laudadio and Tufarelli (2010b) used ISA Brown pullets and 240 g/kg of the faba bean cv. Prothabat.

Before Expt II a faba bean experiment with higher faba bean inclusion was started with laying hens, but it was discontinued after the first four weeks due to high mortality. The successfully completed study (II) was started after 15 weeks discontinued study. The mortality in discontinued study (calculated after the first four-week period) increased 0%, 3.8%, 31.9%, 38.0% with the increased faba bean inclusions of 0 g/kg, 123 g/kg, 247 g/kg, and 371 g/kg, respectively. According to the autopsy reports by the Finnish Food Safety Authority, the cause of death was aplastic anemia. No

other reasons were found. The hens were in a normal nutritional state, but their digestive tracts were almost empty. The hens were extremely anemic. Some of the deceased hens had blood in the peritoneal cavity and blood filled follicles in the ovaries. The hens' spleens were small. Their bone marrows were aplastic; histological analysis showed that the cell counts in the bone marrow were low. The findings in discontinued study support the results of Halle (2005), who discontinued a faba bean study due to high laying hen (LSL classic) mortality in feeding treatments with the faba bean cv. Scirocco at a level of 200 g/kg and the cv. Gloria at a level of 300 g/kg. In line with V + C content determined in this study (II, IV), Halle (2005) also reported high V + C contents for the faba beans studied (cv. Scirocco 9.57 g/kg DM and cv. Gloria 9.81 g/kg DM). According to Castanon and Perez-Lanzac (1990), faba bean inclusion should be restricted in most laying hen diets, and especially in the diets of high-performance hybrid leghorn breeds.

Faba bean inclusion of up to 240 g/kg had no effect on broiler mortality (IV), which supports the findings of Farrell et al. (1999), Nalle et al. (2010) and Gous (2011), who studied faba bean inclusion levels ranging from 200 to 360 g/kg and found no effect on mortality. The mortality rates (II, IV) indicate that the cv. Kontu contained levels of V + C harmful for layers but not for broilers.

Human carriers of a widespread genetic defect experience faba bean toxicity. This is caused by a deficiency of the erythrocyte-located glucose-6-phosphate dehydrogenase (G6PD) (Crépon et al., 2010). To my knowledge, there is no evidence in the literature that this genetic defect also exists in poultry, but this possibility can't be excluded. However, it is conceivable that variations in the V + C content of faba beans and genetic differences of birds may explain the birds' variable tolerance of faba beans.

4.8 DIGESTIBILITY AND ENERGY VALUES OF GRAIN LEGUMES

The CAIDs of proteins and AAs in grain legumes measure in this study (V) together with those reported in the literature are presented in Table 6. The CAIDs of CP were higher ($P \leq 0.05$) in peas and lupin than in the faba bean cv. Kontu (V). However, there was no difference ($P > 0.05$) in the CAIDs of CP in the faba bean cv. Ukko and other legumes. In general, the CAID values of AAs followed the pattern shown by the CAID of protein, and so the CAIDs of AAs were the highest for peas and lupin and the lowest for the faba bean cv. Kontu (V). However, in most cases, the CAIDs of the AAs of the faba bean cv. Ukko did not differ from those of other legumes. In most of cases, when amino acid content in test legume was high the CAID of amino acid was high as well.

The AME values of legumes were in line with their CAIDs of dry and organic matter for faba beans and lupin. However the CAID of dry and organic matter within pea cultivars did not differ, but the AME of pea cv. Karita was 1.6 MJ/kg DM better than that of pea cv. Sohvi. The CAIDs of DM, organic matter, and ash of lupin were lower ($P \leq 0.05$) than those of peas and faba beans. The AME values of grain legumes measured in the present study (V) together with those reported in the literature are presented in Figure 2. Because nitrogen retention is dependent on complete feed, only AME values are presented for grain legumes (V). The AME of the pea cv. Karita was higher ($P \leq 0.05$) than that of the pea cv. Sohvi and of both the faba bean cv. Kontu and cv. Ukko. Lupin had the lowest AME ($P \leq 0.05$).

The high energy value of peas, but also the relative high energy values of faba beans found, indicate that pea and faba bean starches were well digested. However, the major limitations of study (V) were that the composition of dietary fibre was not determined and starch and tannin contents were not analyzed. These measurements would help us interpret differences in AME and CAIDs.

4.8.1 Digestibility and energy value of peas

Lysine, methionine, and threonine in peas were well digested, but semi-essential cysteine (which is a limiting factor in poultry nutrition together with lysine and methionine) was averagely digested in particularly in cv. Karita (V). The CAIDs of CP and AAs of peas were comparable to or even better than those presented in the literature for SBM (Huang et al., 2006; de Coca-Sinova et al., 2008). The CAIDs of AAs in peas were in line with the findings of Nalle et al. (2011b) but approximately 10 %-units higher than reported by Hew et al. (1997) for field peas. There seems to be a lack of studies in which the digestibilities of garden and field peas are compared, but the difference in digestibility is most likely due to differences in tannin content. Field peas have colored flowers (Castell et al., 1996) and usually contain more tannins than garden peas, which are white-flowered (Bastianelli et al., 1998; Smulikowska et al., 2001). In addition to tannins, fibrous material, trypsin- and protease inhibitors, and lectins typically decrease CP availability (Gatel, 1994; Palander et al., 2006). In white-flowered peas, trypsin inhibitors may usually be both the most varying and the most limiting factor (Smulikowska et al., 2001). In the present study, the CP of peas was however well digested, and there was no evidence that trypsin inhibitors would have been a problem.

The AME values of green coloured pea seeds in the present study (V) were considerably higher than reported by Nalle et al. (2011b) (11.7 MJ/kg) for brown or yellow seeded cultivars, but it

remains unclear whether the difference is due to seed colour. Nalle et al. (2010), Nalle et al. (2011a), and Nalle et al. (2011b) showed that the AME_N values of grain legumes are approximately 1 MJ/kg lower than their AME values. Hence the present findings support the results of Smulikowska et al. 2001, who reported an AME_N value of 12.9 MJ/kg DM for white-flowered peas.

4.8.2 Digestibility and energy value of faba beans

Faba bean lysine and threonine were well digested (V). Faba bean methionine was averagely digested, whereas faba bean cysteine was poorly digested. The CAIDs of faba bean methionine and cysteine were lower and the CAIDs of other faba bean AAs and of faba bean CP were slightly lower than reported by Nalle et al. (2010) and Gous (2011), with the exception of the CAID of lysine, which was higher than reported by Gous (2011). The poorer CAIDs detected in present study are most likely explained by the high tannin content of the faba beans used. Nalle et al. (2010) and Gous (2011) used cultivars with low tannin contents. The poor digestibility of AAs containing sulfur is usually the cause of the need for additional crystalline methionine in poultry diets containing legumes reported by Gatel (1994), Steinfeldt et al. (2003), and Nalle et al. (2011a).

The better CAIDs of CP and AAs of the cv. Ukko than those of the cv. Kontu (V) however indicate that cv. Ukko contains less tannins than the cv. Kontu, which is a crossbreed of Ukko and Icarda 536. The results of Lacassagne et al. (1988), Wiseman and Cole (1988), Ortiz et al. (1993), and Vilariño et al. (2009) showed that the digestion of faba bean protein and AAs by chicks was decreased by the level of tannins in the diet. Ortiz et al. (1993) summarized that this may be explained by condensed tannins decreasing the digestibility of dietary protein but of not a single AA. V + C had no effect on nitrogen digestibility in the study Vilariño et al. (2009). The data from this study suggests that the cv. Kontu (hulls) contained sufficient amounts of tannins to decrease the digestibility of protein and AAs to a significantly lower level than that of peas and lupin seeds. In faba beans, the detrimental effect of the tannin content of the hull fraction has been well demonstrated and extensively studied (Longstuff and McNab, 1991; Longstuff et al., 1991; Gatel, 1994). Tannins may lead to an increasing secretion of endogenous proteins and reduce the protein digestibility (Gatel 1994).

The AME in the cv. Ukko (V) was in accordance with the results of Hughes and Choct (1999) and Nalle et al. (2010) (11.0 – 11.5 MJ/kg and 8.8 – 12.0 MJ/kg and, respectively). According to Crépon et al. (2010), the AME_N value of pelleted faba beans in diets for cockerels ranged from 11.2

to 12.5 MJ/kg DM. The AME in the faba bean cv. Kontu was higher than found by Nalle et al. (2010) and Hughes and Choct (1999).

In the case of the low-fat legumes peas and faba beans, starch content and digestibility is of major importance for their energy value (Wiseman and Cole, 1988). Starch digestibility is affected by the polysaccharides of the seed hulls (Longstuff and McNab, 1991). Unlike the situation with protein digestibility, it has been observed that starch digestibility and AME do not always significantly suffer from pea and faba bean tannin content (Lacassagne et al., 1988; Wiseman and Cole, 1988; Vilariño et al., 2009). Very few studies have assessed the impact of V + C on nutrient digestibility in faba beans. However, Vilariño et al. (2009) found a negative effect of V + C on the AME of faba beans in broiler diets.

4.8.3 Digestibility and energy value of lupin

Lysine and threonine in lupin were well digested, whereas methionine and cysteine in lupin were averagely digested (V). The CAID of methionine in the present study was lower than found by Ravindran et al. (2002) and Nalle et al. (2011a), and the CAIDs for threonine and cysteine were lower than found by Nalle et al. (2011a). Otherwise, the CAIDs were in accordance with Ravindran et al. (2002), Nalle et al. (2011a), and Kaczmarek et al. (2014). The differences in CAIDs are most likely due to the use of a different cultivar and their differences in AA contents. Ravindran et al. (2002) found a higher methionine content, and Nalle et al. (2011a) found higher methionine, threonine, and cysteine contents than what were found in the current study.

Although the lupin contained a higher amount of fat than the peas and faba beans, it was the poorest source of AME (V). Lupin's thick hull layer and poor starch content explain its low energy value (Petterson, 2000). In addition, cell wall related material (non-storage polysaccharide fibers) presents at high levels in lupin, especially in *Lupinus angustifolius* (Wiseman and Cole, 1988; Glada, 1998). Cell wall related material is generally assumed to be of negligible nutritive value for poultry due to a low amount of bacterial activity in the hind gut (Wiseman and Cole, 1988; Glada, 1998). The very low AME value of lupin was in line with Olkowski et al. (2001), Petterson et al. (2000), and Nalle et al. (2011a). The AME of lupin kernels has been reported to be higher than that of lupin seeds (Hughes and Choct, 1999).

Table 6. The CAIDs of CP and EAAs and semi-EAAs in grain legume seeds (V) in comparison to literature values.

V											
Pea			Faba bean			Lupin			Hew et al. Nalle et al. (1997)		
cv. Solvi	cv. Karita	Peas	cv. Kontu	cv. Ukko	Faba bean	cv. Pershatsvet	Field pea	(2011b) ¹	(2010) ¹	Gous (2011)	Ravindran et al. (2002)
cv. Solvi	cv. Karita	cv. Karita	cv. Kontu	cv. Ukko	cv. Pershatsvet	cv. Pershatsvet	Field pea	(2011b) ¹	(2010) ¹	Faba bean	Lupin
CP	0.869	0.850	0.771	0.821	0.836	0.836	0.73	nd	nd	nd	0.824
Arginine	0.943	0.922	0.879	0.916	0.931	0.931	0.84	0.915	0.902	0.787	0.903
Cysteine	0.768	0.671	0.458	0.516	0.768	0.768	nd	0.645	0.563	0.659 ⁴	0.688
Histidine	0.888	0.881	0.807	0.835	0.860	0.860	0.74	0.816	0.716	nd	0.837
Isoleucine	0.868	0.861	0.793	0.845	0.868	0.868	0.70	0.840	0.829	0.879	0.817
Leucine	0.890	0.872	0.817	0.874	0.881	0.881	0.70	0.844	0.836	0.871	0.837
Lysine	0.924	0.919	0.869	0.900	0.891	0.891	0.71	0.888	0.894	0.750	0.780
Methionine	0.899	0.858	0.714	0.791	0.794	0.794	0.71	0.817	0.812	0.780	0.828
Phenylalanine	0.872	0.859	0.761	0.838	0.848	0.848	0.71	0.874	0.884	0.885 ³	0.830
Threonine	0.852	0.831	0.770	0.811	0.796	0.796	0.68	0.779	0.770	0.846	0.762
Tyrosine	0.784	0.809	0.728	0.790	0.763	0.763	nd	0.810	0.805	nd	0.848
Valine	0.879	0.893	0.847	0.845	0.888	0.888	0.69	0.836	0.815	nd	0.796
Overall mean ²	0.880	0.860	0.773	0.826	0.848	0.848	nd	0.825	0.796	nd	0.814
Mean of four cultivars											
² Mean of all amino acids											
³ CAID of phenylalanine+tyrosine											
⁴ CAID of methionine+cysteine											
⁵ Mean of three cultivars											
Nalle et al. (2011a) ⁵			Nalle et al. (2014) ¹			Nalle et al. (2011a) ⁵			Nalle et al. (2014) ¹		
Lupin			Lupin			Lupin			Lupin		
0.809			0.809			0.809			0.809		
0.839			0.839			0.839			0.839		
nd			nd			nd			nd		
0.764			0.764			0.764			0.764		
0.829			0.829			0.829			0.829		
0.844			0.844			0.844			0.844		
0.823			0.823			0.823			0.823		
nd			nd			nd			nd		
0.830			0.830			0.830			0.830		
0.761			0.761			0.761			0.761		
0.779			0.779			0.779			0.779		
0.798			0.798			0.798			0.798		

¹Mean of four cultivars

²Mean of all amino acids

³ CAID of phenylalanine+tyrosine

⁴ CAID of methionine+cysteine

⁵Mean of three cultivars

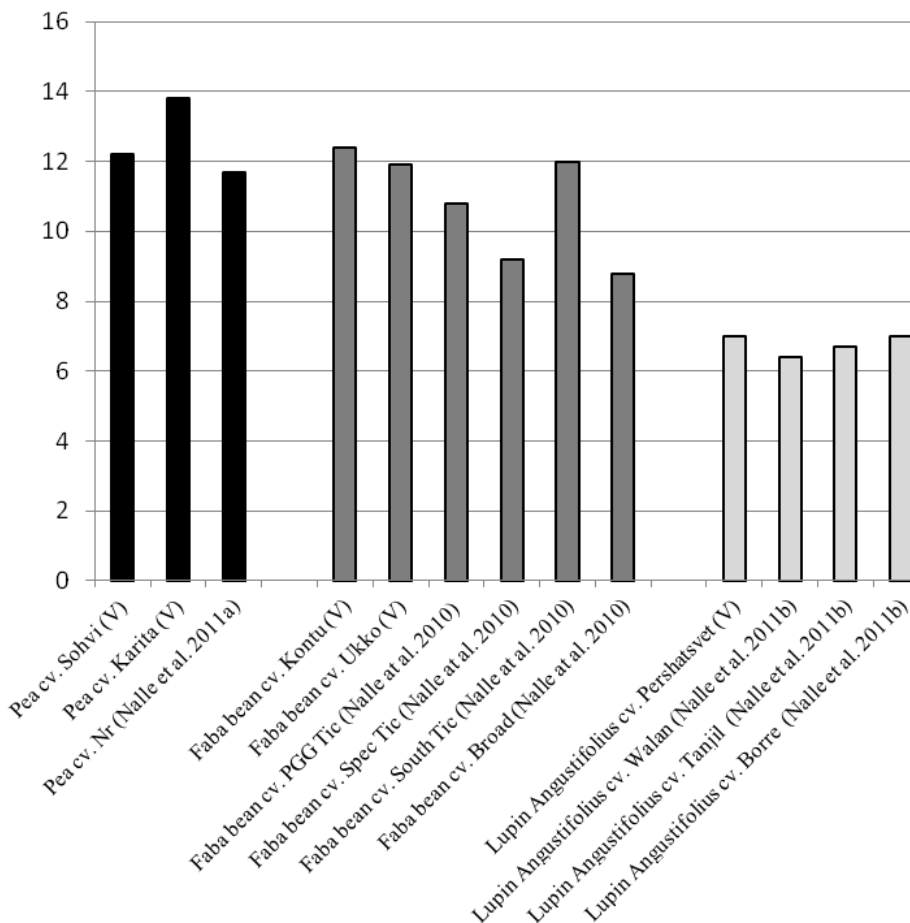


Figure 2. The AME values (MJ/kg DM) of grain legume seeds (V) in comparison to literature values.

Nr = not reported

4.9 DIGESTIBILITY AND ENERGY VALUES OF FABA BEAN DIETS

The AME_N value of the diets increased ($P \leq 0.05$) along faba bean inclusion (IV). Because the faba bean cv. Kontu is only a moderate source of energy (V), the higher AME_N of the diet with faba bean inclusion in Expt IV is most likely due to the higher amounts of rapeseed oil and wheat used together with faba bean inclusion. The well digested AAs in faba beans may have, however, had a positive effect on increased nitrogen retention and hence the AME_N value. The findings of the

present study support the results of Palander et al. (2006), which showed that the AME_N in a cereal–SBM based diet for 5-week-old turkeys was considerably lower than in a faba bean diet.

The CAID of alanine, arginine, asparagine, histidine, and threonine increased ($P \leq 0.05$) in a linear manner with increased faba bean inclusion (IV). This is most likely because there was less wheat in the diet as the faba bean inclusion increased. The digestibilities of these AAs have been shown to be good in faba beans (Nalle et al., 2010). The good digestibility of most AAs, particularly alanine, arginine, asparagine, and histidine, indicated that the AAs in faba beans were digested as well as those in SBM and better than those in wheat. This supports the ileal digestibility values presented in the literature for wheat (Lemme et al., 2004). The digestibility of cysteine decreased in a linear manner with higher faba bean inclusion (IV) due to the poor digestibility of the cysteine in faba beans (V). The data suggests that tannins have no negative effect on diet protein or AA digestibility and the AME_N value when up to 240 g/kg of faba beans are used in diets.

5 CONCLUSIONS

1. The protein content of grain legumes is lower than that of SBM. However, the protein of grain legumes can partially replace the protein of SBM in poultry diets. Due to high energy content of especially pea, but also faba bean, they also partially replace the energy of cereals in the diets. In general, compared to SBM protein, all legume proteins were a good source of lysine. Because legumes are deficient in methionine and because their cysteine digestibility is low especially in faba beans, diets containing legumes should be enriched with feed-grade crystalline methionine to avoid a decrease in performance. In addition, feed-grade crystalline lysine is also usually needed. The necessary amount of AA additions is dependent on the protein and AA contents of the feed ingredients used and the desired protein and AA contents.

2. Because peas are a good source of AME, and the AAs of peas are well digested, they can be used in relatively high amounts in poultry diets. At least 300 g/kg of semi-leafless peas (cv. Karita) can be used in laying hen diets, whereas at least 450 g/kg of these peas can be used in broiler diets provided that diets based on cereal and SBM is used. The use of peas in diet may, however, not be restricted by the inclusion levels determined in this study, but other factors such as the cost of feed, the nutrient requirements of birds or the desired cereal proportion in the diet may restrict the use of peas in poultry diets.

3. Because faba beans contain more ANFs than peas, are moderate sources of AME and their AAs are averagely digested, their use in poultry diets is more restricted than that of peas. It is recommended to use the tannin and V + C containing faba bean cv. Kontu at inclusion levels of at most 50 g/kg in laying hen diets and 160 g/kg in broiler diets based on cereals. However, SBM should be used beside faba bean inclusion. As the protein content of faba bean is higher than that of pea, the same amount of faba beans can replace more SBM protein in poultry diets than peas.

4. The mortality rates indicate that faba bean cv. Kontu contains levels of V + C harmful for layers but not for broilers. It should be further studied whether genetic differences of birds would explain the birds' variable tolerance of faba beans.

5. The use of the enzyme cocktail of XLS, AMS, and PRT improves the nutritive value of wheat-pea diets and decreases viscosity in ileal digesta as shown by the improved performance of broilers. The beneficial effects of this are, however, more likely a result of the effects of the enzymes on backbone substituent in wheat. The enzyme cocktail of XLS, AMS, and PRT is not applicable to improve nutritive value of pea.

6. Most AAs, especially lysine, are well digested in the blue lupin cv. Pershatsvet. Lupin is a poor source of AME. The poor energy value of blue lupin is related to its poor apparent ileal digestibility of dry and organic matter most likely due to NSPs. In addition, the use of exogenous enzymes could improve the energy value of lupines.

6 FUTURE PERSPECTIVES AND PRACTICAL IMPLICATIONS

At the time of the experiments, the pea cv. Karita and the faba bean cv. Kontu were the most cultivated pea and faba bean cultivars in Finland. They still dominate the market, but newer pea cultivars like Hulda, Rocket, and Ingrid are increasing their share. In the faba bean market, the new cultivars Sampo and Louhi are joining the cv. Kontu. However these newer cultivars were not primarily bred to provide better nutrition values for livestock (Pärssinen personal communication). The pea cv. Karita (growth time 99 days and yield 3967 kg per hectare) and the faba bean cv. Kontu (growth time 144 days and yield 3200 kg per hectare) are harvested as seeds in southern Finland (in the growing zones I and II). The pea cv. Karita can also be cultivated in the growing zone III.

The faba bean cultivar Kontu has high tannin and V + C contents, and therefore the inclusion level of this cultivar recommended by this study (50 g/kg in laying hen diet and 160 in broiler diet) is lower than the recommendations for faba beans in the literature (in many studies 200 g/kg in laying hen and broiler diets). Faba beans low in tannin and V + C are already available (cv. Disco in France, released 2003), and because they are almost devoid of ANF, they are more suitable for incorporation into poultry feeds. However, the faba bean cultivars Kontu and Ukko have the greatest potential for being cultivated in Finland. Cultivars available on the market that are low in tannin and V + C contents are not adapted to a short growing season and are therefore not commercially cultivated in Finland. However, one breeding goal for faba bean is to produce less ANFs containing faba beans.

There seems to be a lack of data comparing the digestibility of AAs and energy values of white and coloured flower pea cultivars and of garden and field peas. In the future, it would be of interest to study whether any differences exists. Most pea cultivars in Finland have green seeds. However, cultivars with yellow and brown seeds are also cultivated. It would be interesting to study whether yellow or brown seed cultivars have any advantages to green seed cultivars. However, potential and realities of cultivating those coming varieties on a large scale should be consider before the intention to incorporate them to Finnish poultry diets.

It is essential to maintain faba bean breeding programmes to develop cultivars without tannin and V + C for the Finnish climate. In the future, it will be of interest to evaluate the nutritional values of future faba bean cultivars adapted to cultivation in Finland for use in poultry diets. There is a lack of studies on the potential impact of the genetic differences of birds on their ability to tolerate faba beans. From a scientific point of view, this would be very interesting and a novel subject in research on the use of faba beans in poultry nutrition. It would also be of interest to study more locally cultivated *Lupinus* species used in poultry nutrition. It would be of benefit to find their appropriate inclusion levels. Moreover, there is a need to breed lupin cultivars to adapt them to the Finnish climate.

The results of this thesis show that grain legumes can be used successfully in poultry diets. One promising way to increase protein self-sufficiency is to increase the cultivation of grain legumes used in poultry feeds. The results of this thesis increase awareness of the nutrition values of grain legumes for poultry and the appropriate inclusion levels of Finnish pea and faba bean cultivars in poultry diets. This knowledge is important for people working in the feed industry and expert organizations and, last but not least, for farmers. Legumes have an important role in organic production. Demand for alternative protein sources in organic production will increase even more when the exemption in organic productions regulations allowing use of up to 5% conventional protein feed in piglets and poultry ends in 2018. When grain legumes are included in a diet, they occupy space at the expense of both grain (cereals) and protein (SBM) ingredients. So, how economical the use of grain legumes in poultry feeds is, depends on their prices and the prices of the other feed ingredients (cereals and SBM).

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